

# Influence of *Pediococcus*-Based Probiotic on Coccidiosis in Broiler Chickens

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**ABSTRACT** Coccidiosis is the major parasitic disease of poultry and is caused by the apicomplexan parasites *Eimeria*. Drugs and live vaccines are the 2 main control measures of the disease; however, due to increasing concerns with prophylactic drug use and the high cost of vaccines, alternative control methods are needed. Recent evidence that various dietary and live microbial supplements can influence host immunity against enteric diseases prompted us to investigate the role of a *Pediococcus*-based probiotic on coccidiosis in broiler chickens. In the present study, we examined BW gains, oocyst shedding, and antibody responses of broilers fed the commercial probiotic MitoGrow. Day-old chicks were fed either a regular broiler diet or 1 of 2 probiotic diets supplemented with 0.1% (MG 0.1) or 0.2% MitoGrow. Chicks were orally

challenged with 5,000 or 10,000 sporulated oocysts of *Eimeria acervulina* or with 5,000 *Eimeria tenella* oocysts on d 10 or 12 of age, respectively. In *E. acervulina*-infected birds, the MG 0.1 group improved ( $P < 0.05$ ) weight gain as compared with the other 2 groups and reduced ( $P < 0.05$ ) oocyst shedding in birds infected with 5,000 *E. acervulina* oocysts. In *E. tenella*-infected birds, *Eimeria*-specific antibody levels were higher ( $P < 0.05$ ) in the MitoGrow-fed groups, especially in the MG 0.1 birds, compared with the regular diet group, although their oocyst shedding and weight gains were not clearly improved. These results demonstrate that this *Pediococcus acidilactici*-based probiotic effectively enhances the resistance of birds and partially protects against the negative growth effects associated with coccidiosis, particularly when supplemented at 0.1% MitoGrow of the diet.

**Key words:** *Pediococcus*, probiotic, coccidiosis, broiler, antibody

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## INTRODUCTION

Avian coccidiosis is the major parasitic disease of poultry causing mortality, malabsorption, inefficient feed utilization, impaired growth rate in broilers, and reduced egg production in layers (Lillehoj et al., 2004). The disease presents tremendous economic significance to the poultry industry, with an estimated worldwide annual loss of more than \$3 billion (Williams, 1999; Dalloul and Lillehoj, 2006). Currently, drugs and live vaccines are the 2 main control measures for the disease; however, due to increasing problems with prolonged drug usage and the high cost of vaccines, alternative strategies are needed for more effective and safer control of coccidiosis in chickens (Dalloul et al., 2006; Williams, 2006).

Recent evidence that various dietary and microbial supplements can influence host immunity against enteric diseases prompted us to investigate the role of a commercial probiotic (MitoGrow, Imagilin Technology LLC) on coccidiosis. This probiotic consists of live *Pediococcus acidilactici*, which belongs to the homofermentative gram-positive bacteria, able to grow in a wide range of pH, temperatures, and osmotic pressures, and thus able to colonize and inhabit

the digestive tract (Klaenhammer, 1993). Some commercial bacteria have been found to enhance development of both the intestinal epithelia and the gastrointestinal lymphoid system (Falk et al., 1998; Umesaki and Setoyama, 2000). A balanced microbial population would support the inherent defense mechanisms of a healthy intestinal tract, resulting in better control of intestinal pathogens (Pollmann et al., 2005). *Pediococci* exert antagonism against other microorganisms, including enteric pathogens, primarily through the production of lactic acid and secretion of bacteriocins known as pediocins (Daeschel and Klaenhammer, 1985). Guerra et al. (2006) suggested that the nonpathogenic and nontoxic bacterium *Pediococcus acidilactici* induces healthy intestinal conditions in pigs. We hypothesized that MitoGrow, containing *P. acidilactici*, may interfere with the pathogen infection sites, produce antimicrobial peptides, or induce host immune responses, thus enhancing its resistance to enteric pathogens like *Eimeria*. In the present work, 2 trials were conducted to investigate the potential protective effects of the probiotic MitoGrow in broiler chickens experimentally infected with *Eimeria acervulina* or *Eimeria tenella*.

## MATERIALS AND METHODS

### Experimental Designs

**Experiment 1.** In the initial study, 70 day-old broiler chicks were used to evaluate the protective effects of feed-

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ing MitoGrow against *E. acervulina*. Broilers were randomly assigned to 7 pens ( $n = 10/\text{pen}$ ) of an electrically heated battery and were fed a regular nonmedicated broiler starter diet either without a probiotic (30 birds; **REG**) or with the probiotic MitoGrow supplemented at the rate of 0.1% (**MG 0.1**) or 0.2% (**MG 0.2**) of the diet (20 birds each). At 10 d of age, 10 birds from each group were inoculated with either 5,000 or 10,000 sporulated *E. acervulina* oocysts (total of 30 birds for each inoculation rate), whereas the remaining 10 birds of the REG diet served as negative controls and were placed at 2 birds/cage (5 cages/treatment). Body weights were measured at 0 and 10 d postinoculation (**dpi**), and fecal samples were collected from 5 cages from 6 to 9 dpi.

**Experiment 2.** In the second trial, 120 day-old broilers were randomly assigned to 12 pens ( $n = 10/\text{pen}$ ) of an electrically heated battery and were equally assigned to 1 of 3 experimental diets (4 pens/diet group): REG, MG 0.1, or MG 0.2, as above. At 12 d of age, half of the birds from each diet group ( $n = 20$ ) were then orally inoculated with 5,000 *E. tenella*-sporulated oocysts, and 10 birds from each treatment were placed at 2 birds/cage (5 cages/treatment) as above. In addition to measuring weight gains and oocyst shedding (6 to 10 dpi), serum samples were collected 10 dpi, and *Eimeria*-specific antibody (**Ab**) titers were determined by ELISA.

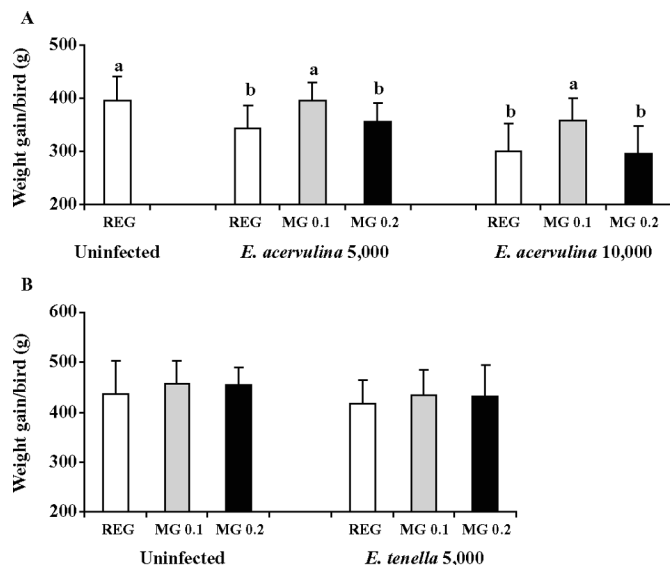
All diets were formulated to meet or exceed the nutrient requirements for broilers as recommended by the NRC (1994). Feed was provided ad libitum, and the animal trials were performed according to the guidelines established by the Beltsville Area Institutional Animal Care and Use Committee.

## Oocyst Shedding

Oocyst shedding was assessed as described by Dalloul et al. (2002). Briefly, droppings from 10 birds in 5 cages (2 birds/cage) were collected for 4 to 5 d starting on 6 dpi, fecal material was ground and homogenized, and two 35-mL samples were taken, diluted, and the oocysts were counted microscopically using a McMaster counting chamber. The total number of oocysts was calculated using the following formula: total oocysts/bird = oocyst count  $\times$  dilution factor  $\times$  (fecal sample volume/counting chamber volume)/number of birds per cage.

## ELISA for Serum Ab Levels

Blood samples were obtained 10 dpi from individual birds ( $n = 3/\text{group}$ ), allowed to clot for 4 to 5 h at 4°C, and the sera were collected. Serum samples were tested for *Eimeria*-specific Ab levels using ELISA, as described (Dalloul et al., 2003). Briefly, a microtiter plate was coated overnight with 10  $\mu\text{g}/\text{well}$  of the recombinant coccidial antigen, EtMIC2. The plate was washed with PBS-0.05% Tween and blocked with PBS-1% BSA. Serum dilutions (1:16; 100  $\mu\text{L}/\text{well}$ ) were added, incubated with continuous gentle shaking, washed with PBS-0.05% Tween, and bound Ab detected with peroxidase-conjugated rabbit anti-



**Figure 1.** Body weight gains of broiler chickens fed regular (REG), 0.1 or 0.2% MitoGrow-supplemented diets (MG 0.1 and MG 0.2, respectively). Birds uninfected or infected with 5,000 or 10,000 *Eimeria acervulina* oocysts at d 10 posthatch and kept on the same diets (panel A); birds uninfected or infected with 5,000 *Eimeria tenella* oocysts at d 12 posthatch (panel B). Each bar represents the mean  $\pm$  SD ( $n = 10$  for panel A;  $n = 20$  for panel B). <sup>a,b</sup>Means lacking common letters differ in uninfected or infected chickens ( $P < 0.05$ ).

chicken IgG (Sigma-Aldrich, St. Louis, MO) and peroxidase-specific substrates. Optical density was determined with a microplate reader (BioRad, Richmond, CA) at 450 nm.

## Statistical Analyses

Mean values for BW gains, fecal oocyst shedding, and Ab titers were compared by the Tukey-Kramer multiple comparisons test following ANOVA, using InStat software (GraphPad, San Diego, CA). Differences between means were considered significant at  $P < 0.05$ .

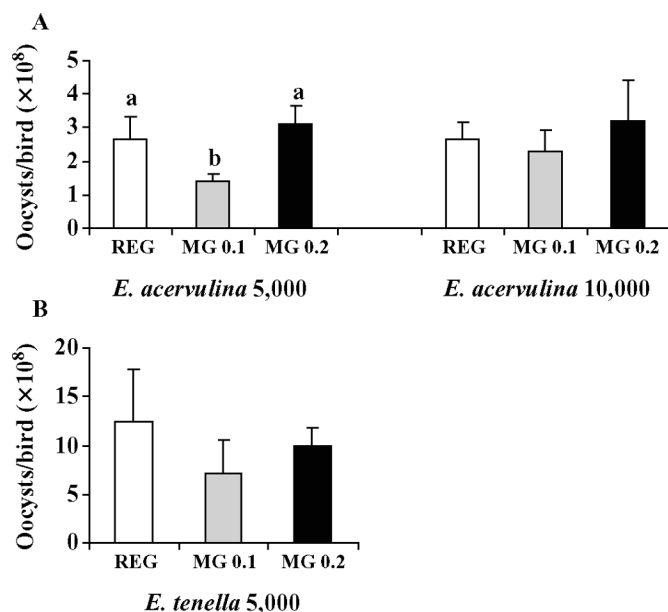
## RESULTS

### BW Gains

Individual BW were taken at 0 and 10 dpi, and mean weight gains of uninfected and *E. acervulina*- or *E. tenella*-infected birds were calculated over the 10-d infection period. As shown in Figure 1, panel A, REG and MG 0.2 groups in the *E. acervulina*-infected birds gained significantly ( $P < 0.05$ ) less weight than the uninfected REG group. However, birds in the MG 0.1 group had higher ( $P < 0.05$ ) weight gains than any of the *E. acervulina*-infected birds and similar gains to the noninfected controls regardless of the infection dose. In the *E. tenella* experiment (Figure 1, panel B), no significant differences were observed among the weight gains of any of the treatments.

### Oocyst Shedding

Figure 2 shows the mean oocyst shedding per bird of *Eimeria*-infected groups on either regular or probiotic-sup-

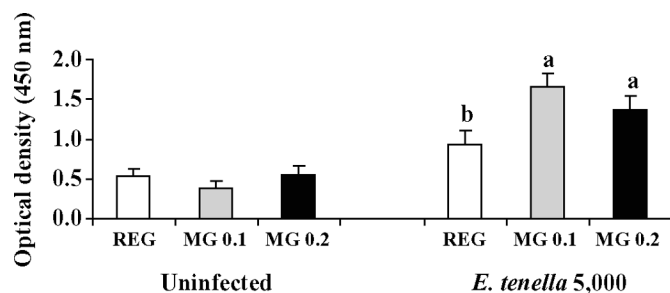


**Figure 2.** Fecal oocyst shedding of broiler chickens fed regular (REG), 0.1 or 0.2% MitoGrow-supplemented diets (MG 0.1 and MG 0.2, respectively). Oocysts counted in fecal material collected 6 to 9 d postinoculation (dpi) with 5,000 or 10,000 *Eimeria acervulina* oocysts at d 10 posthatch (panel A); 6 to 10 dpi by 5,000 *Eimeria tenella* oocysts at d 12 posthatch (panel B). Each bar represents the mean  $\pm$  SD ( $n = 5$ ). <sup>a,b</sup>Means lacking common letters differ in uninfected or infected chickens ( $P < 0.05$ ).

plemented diets. The uninfected control (REG) groups excreted no oocysts. Feeding 0.1% MitoGrow significantly ( $P < 0.05$ ) reduced oocyst shedding in birds infected with 5,000 *E. acervulina* oocysts (Figure 2, panel A). In *E. tenella*-infected groups (Figure 2, panel B), the birds fed MitoGrow diets shed apparently less oocysts than the infected-REG birds, though there was no significant difference between the birds fed regular and probiotic-supplemented diets.

### Serum Ab Responses

To assess Ab responses to *Eimeria* antigen, EtMIC2 was used in this study, and the ELISA results are shown in Figure 3. There was no significant difference in Ab re-



**Figure 3.** Anti-EtMIC2 antibody response of broilers fed nonprobiotic (REG), 0.1 or 0.2% MitoGrow-supplemented diets (MG 0.1 and MG 0.2, respectively) for 21 d. Birds were either uninfected or infected with 5,000 *Eimeria tenella* oocysts at d 12 posthatch, and sera were sampled 10 d postinoculation. Each bar represents the mean  $\pm$  SD ( $n = 3$ ). <sup>a,b</sup>Means lacking common letters differ in uninfected or infected chickens ( $P < 0.05$ ).

sponses to *Eimeria* antigen among all uninfected groups. However, in the 5,000 *E. tenella*-infected and MitoGrow-fed birds, significantly ( $P < 0.05$ ) higher serum *Eimeria*-specific Ab levels were detected when compared with those of birds infected with 5,000 *E. tenella* and fed a regular diet without probiotic.

### DISCUSSION

The present work investigated the effects of a *Pediococcus*-based probiotic (MitoGrow) on susceptibility of chickens to coccidiosis. The most prominent symptom of avian coccidiosis is growth retardation characterized by reduced weight gains or even weight loss in severe cases, causing a major economic effect to the poultry industry (Dalloul and Lillehoj, 2006). MitoGrow-enhanced resistance to experimental *E. acervulina* infection was best exemplified by increased BW gain compared with infected controls, and such protection was further reflected by reduced oocyst shedding, particularly in those birds infected with 5,000 *E. acervulina* oocysts. In *E. tenella*-infected birds, the weight gains or oocyst shedding of the MitoGrow-fed groups were not clearly improved. Although it is desirable to see positive effects with both parameters, direct correlation between increased weight gain and reduced oocyst shedding has not always been the case with probiotic studies (Dalloul et al., 2005). Others have also observed similar results when no correlation between BW gain and oocyst output was found (Talebi and Mulcahy, 1995; Gabriel et al., 2006). Further, the differential effect with the 2 *Eimeria* species tested could be attributed to the species-specific infection sites, where probiotic organisms may favor colonizing 1 site over the other.

In earlier work, Dalloul et al. (2003) reported that administration of a *Lactobacillus*-based probiotic induced protective immunity against *E. acervulina* infection. Some strains of *Pediococcus* species produce antimicrobial peptides (bacteriocins) that inhibit closely related lactic acid bacteria and other gram-positive spoilage and pathogenic bacteria (Klaenhammer, 1993; Ennahar and Deschamps, 2000). These bacteriocins are designated pediocins, and they have been shown to exert high antimicrobial activity against *Listeria* species (Ennahar et al., 2000). *Pediococcus acidilactici* is reported as a nonpathogenic and nontoxic bacterium inducing healthy intestinal conditions in pigs (Guerra et al., 2006). In the current study, we showed for the first time that MitoGrow consisting of live *P. acidilactici* bacteria provided some degree of defense against *E. acervulina* and *E. tenella* infections in broiler chickens.

The microneme protein EtMIC2 was cloned from *E. tenella* (Tomley et al., 1996; Lillehoj and Lillehoj, 2000; Dalloul et al., 2005). Additionally, EtMIC2 represents 1 of nearly 30 *Eimeria* genes that have been cloned and characterized at the molecular level (Allen and Fetterer, 2002). *Eimeria*-specific antibodies to EtMIC2 antigen were significantly ( $P < 0.05$ ) higher in chickens fed the MitoGrow diets in *E. tenella*-infected birds. The role of parasite-specific antibodies has been extensively studied in coccidiosis (Lillehoj and Ruff, 1987; Fayer and Jenkins, 1992; Dalloul et al., 2005).



Although humoral immunity to coccidiosis seems to play a minor function (Dalloul and Lillehoj, 2005, 2006), *Eimeria* infections trigger a significant specific Ab immune response in serum (Dalloul et al., 2005), and immunoglobulins could have a contributory function in the defense of the host against *Eimeria* (Wakelin and Rose, 1990; Lillehoj and Trout, 1996). *Eimeria tenella*-infected birds fed MitoGrow produced more parasite-specific antibodies in the circulation, and these Ab-mediated responses may play a more protective role against a secondary *E. tenella* infection than a single inoculation, as was the case in the present work.

In conclusion, these results demonstrate that a *P. acidilactici*-based probiotic (MitoGrow) enhances the resistance of birds and partially protects against coccidiosis. However, the mechanistic details mediating such protection are not fully understood and remain to be clarified, especially in light of the wide array of immune cells activated by probiotic bacteria. In particular, analysis of the different cytokines and chemokines induced by feeding MitoGrow will provide valuable new information on its protective immunity to coccidiosis. Also, the exact modes of action of this probiotic and its activity against different species of *Eimeria* such as *Eimeria maxima* need to be explored in future work.

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